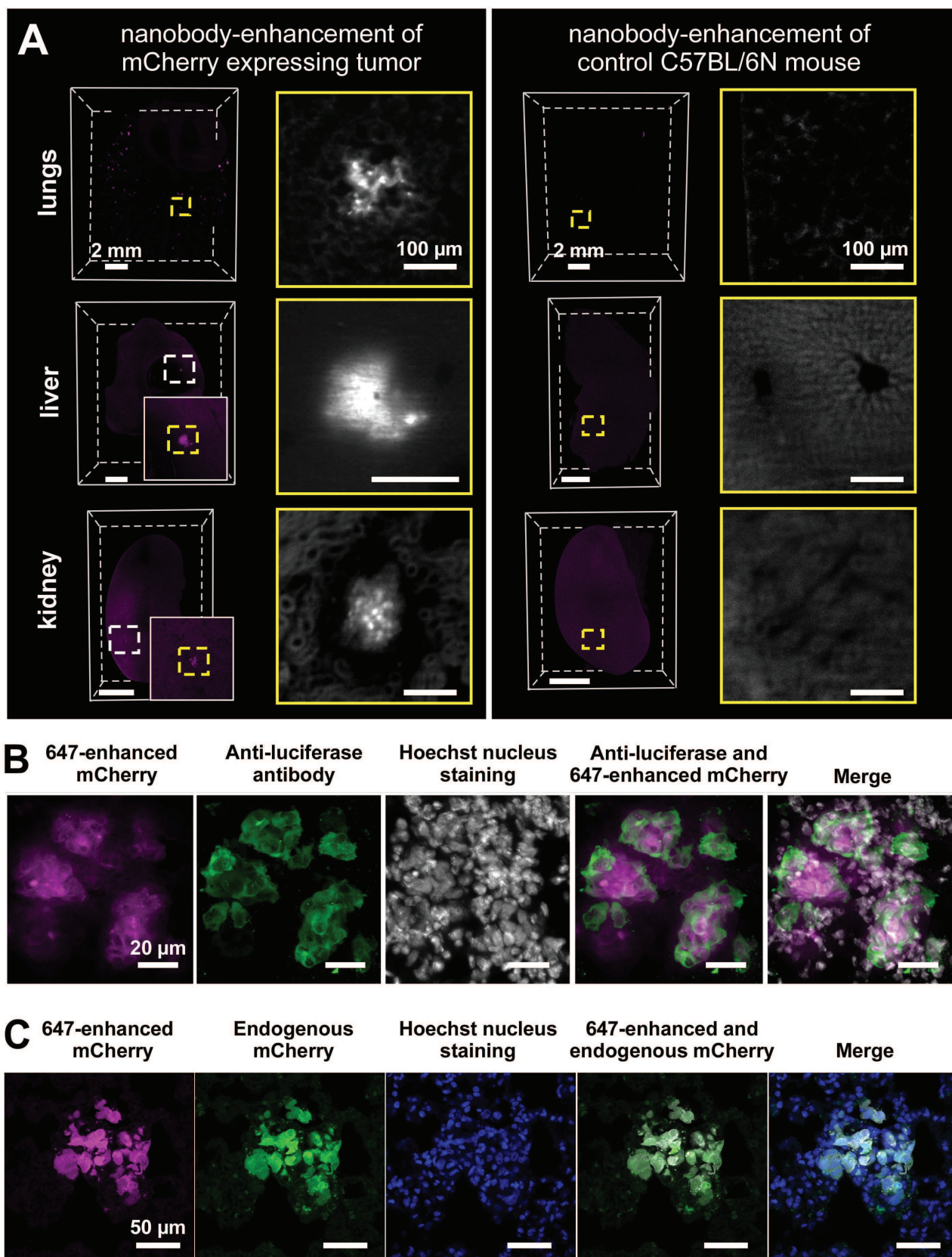


**Figure S1; related to Figure 2**

**vDISCO nanobody enhancement of the fluorescent signal of cancer cells**

(A-C) Representative light-sheet images of mCherry expressing tumor metastases in the lungs of cleared mice that were not enhanced with nanobodies (A), metastases treated with an anti-mCherry nanobody conjugated to Atto594 (B) or an anti-mCherry nanobody conjugated to Atto647N (C). (D) Plots of signal intensity profiles along the yellow lines in panels A-C: non-enhanced mCherry (orange), mCherry enhanced with Atto594 (magenta) or mCherry enhanced with Atto647N (cyan) ( $n=3$  representative metastases). (E) Intensity profiles of the fluorescence signal in (D) normalized over the background. (F-I) 3D light-sheet examples of deep-tissue imaging of Atto647N-enhanced tumor metastases in transparent mice after vDISCO. Tumor micrometastases can be detected (yellow arrowheads) which are located several millimeters deep in the brain (F), liver (G), kidney (H) and spinal cord (I) respectively. (J-L) Examples of micrometastases detected in bone marrow (J), ovary (K) and muscle (L). Note that all the micrometastases shown in G-L were imaged with a 1.1x objective from the MDA-MB-231 tumor model (intracardial injections), except the bone marrow metastasis in (J), which was from the MCF-7 tumor model.



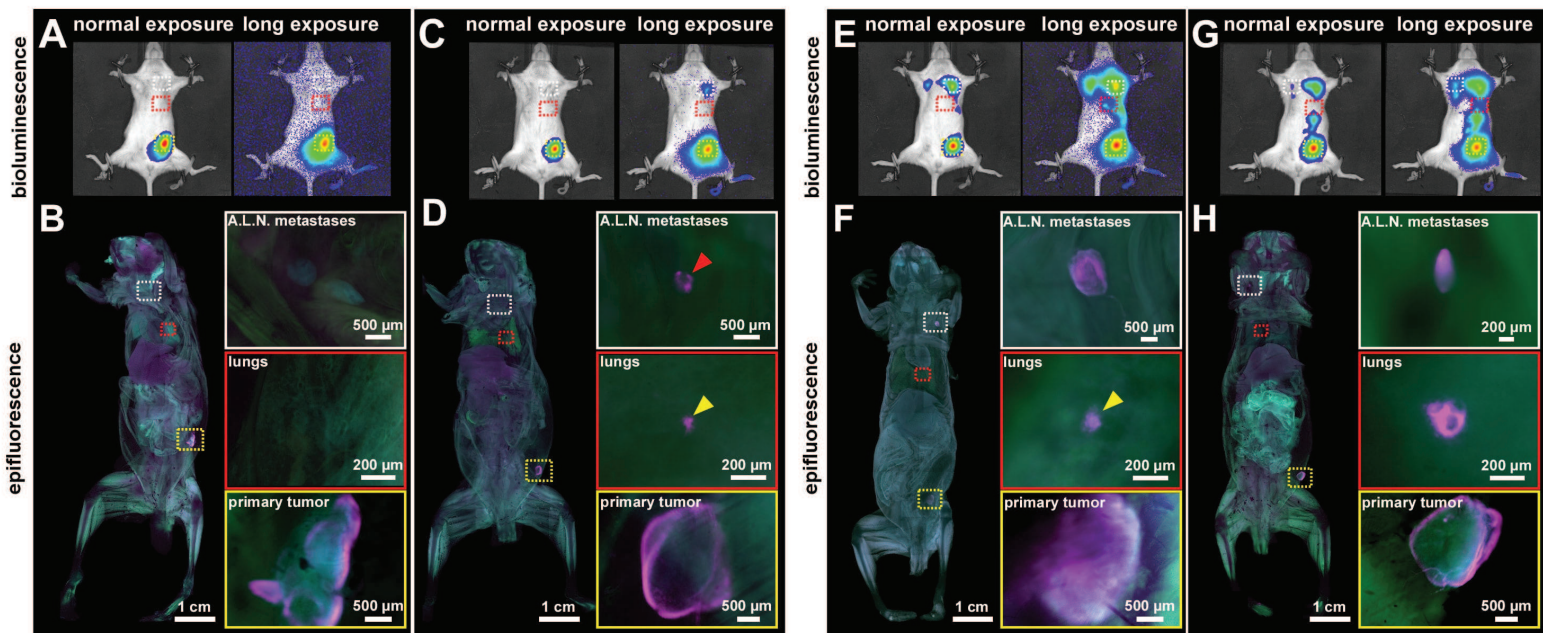
## Figure S2; related to Figure 2

### Confirmation of specificity of nanobody-enhanced staining in mice bearing mCherry-expressing tumors

(A) Comparison between an animal bearing an mCherry expressing tumor and a C57BL/6N control animal, which were both enhanced with an anti-mCherry nanobody conjugated to Atto647N (magenta) and imaged by light-sheet microscopy. No signal is detected in organs from the C57BL/6N control. Note that the background is enhanced to demonstrate the absence of signal in the high-magnification images. (B) Confocal images of metastatic lung tissue immunolabeled with an anti-firefly luciferase antibody (green) after rehydration of the cleared tissue; Atto647N-enhanced cancer cells and cell nuclei are shown in magenta and grey, respectively. (C) Confocal images of a metastasis in the lung of an animal labeled with an anti-mCherry nanobody conjugated to Atto647N. The enhancing nanobody is shown in magenta, mCherry is shown in green and cell nuclei are shown in blue indicating that nanobody-enhancement specifically detects mCherry.

Low tumor load mice with few metastases in bioluminescence

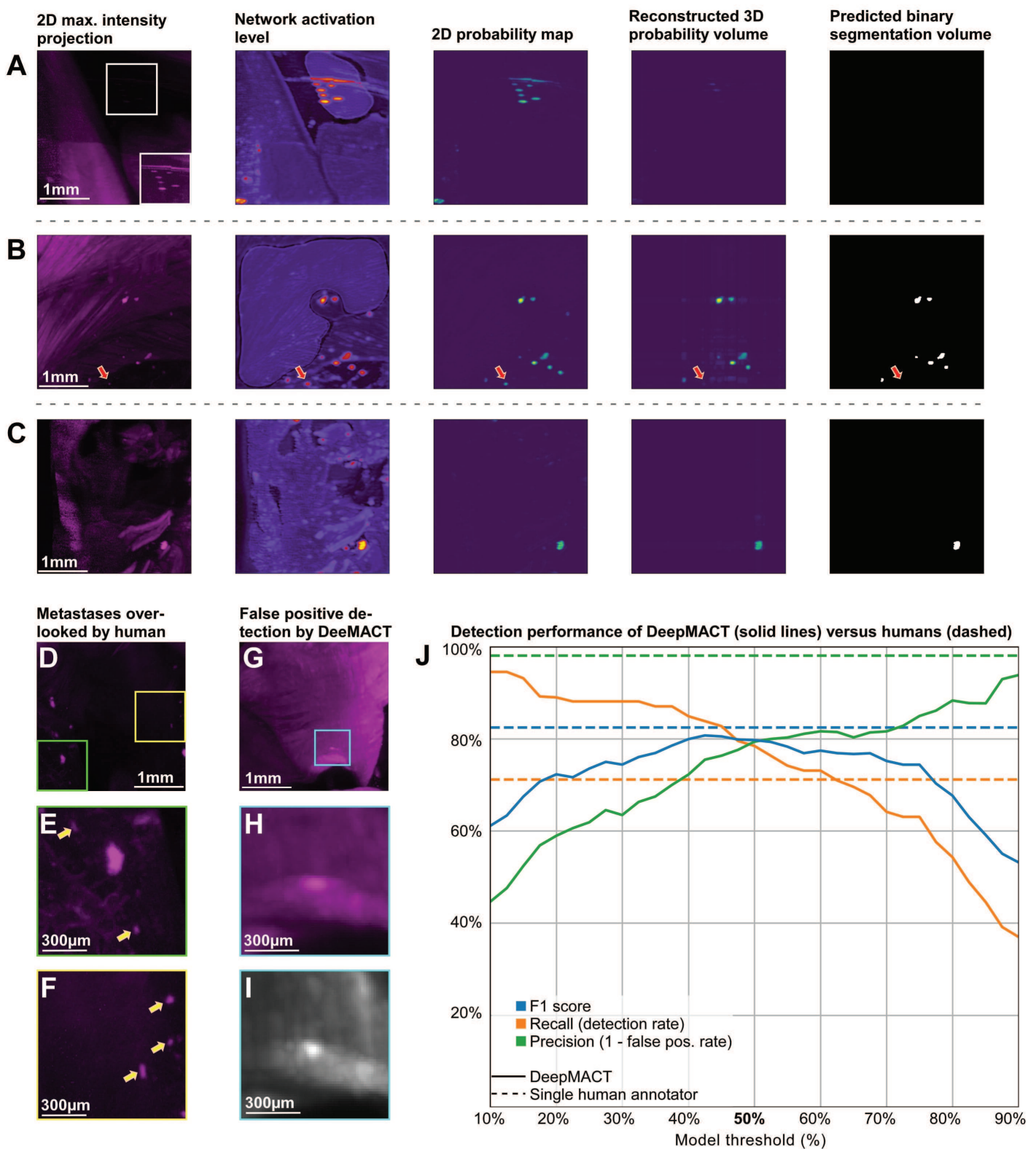
High tumor load mice with several metastases in bioluminescence

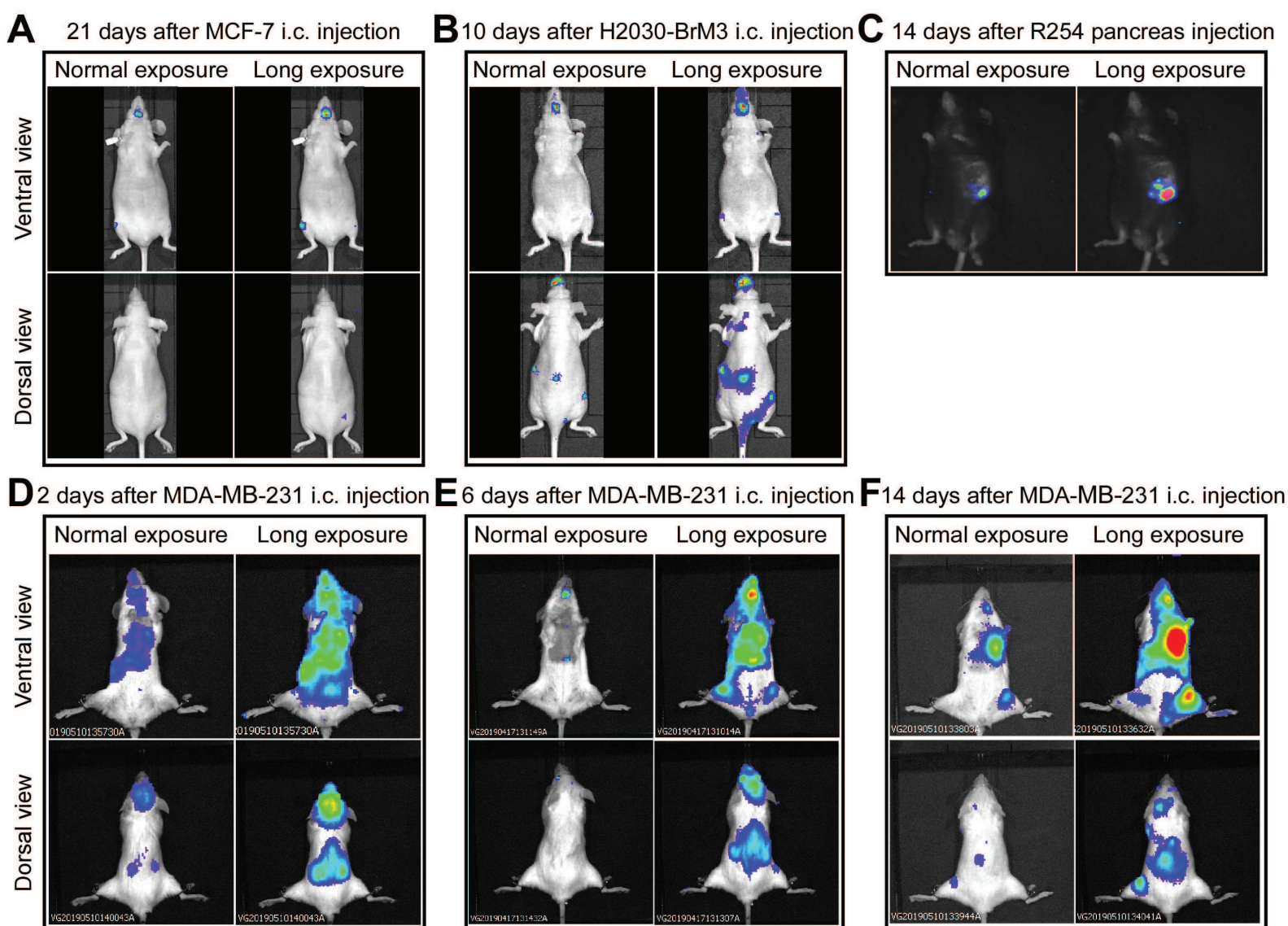


**Figure S3; related to Figure 2**

**Examples of tumor metastasis detection in mice using bioluminescence imaging versus vDISCO and epifluorescence microscopy**

Mice were transplanted in the mammary fat pad with MDA-MB-231 cells and imaged with bioluminescence followed by vDISCO clearing and epifluorescence imaging. (A-D) We found that bioluminescence imaging with normal exposure is not sufficiently sensitive to detect all the metastases in low tumor load mice. For example, the mice in (A,B) and (C,D) had very similar bioluminescence images with normal exposure. Applying vDISCO to these mice, we found no tumor metastases in one case (A,B) and a large metastasis (red arrowhead) in the axillary lymph node (A.L.N. metastasis) (C,D) using a fluorescence stereo microscope. Although the signal from the primary tumor is strong in both normal and long exposure bioluminescence images (A,C, yellow box), metastases in lungs (A,C, red boxes) are not visible, but are detected by epifluorescence imaging (D, yellow arrowhead). In epifluorescence images, the tumors (A647N labeled) are shown in magenta and the background, imaged at 488 nm, is shown in green. (E-H) In mice with high tumor load, a bulk heat map of metastatic distribution can be obtained by bioluminescence imaging, without detailed shape and size information. In contrast, vDISCO resolved single micrometastases in mouse bodies even with a fluorescence stereo microscope. Especially in the lungs, even micrometastases with a diameter smaller than 100  $\mu\text{m}$  could be resolved in full body scans of mice (F, yellow arrowhead).

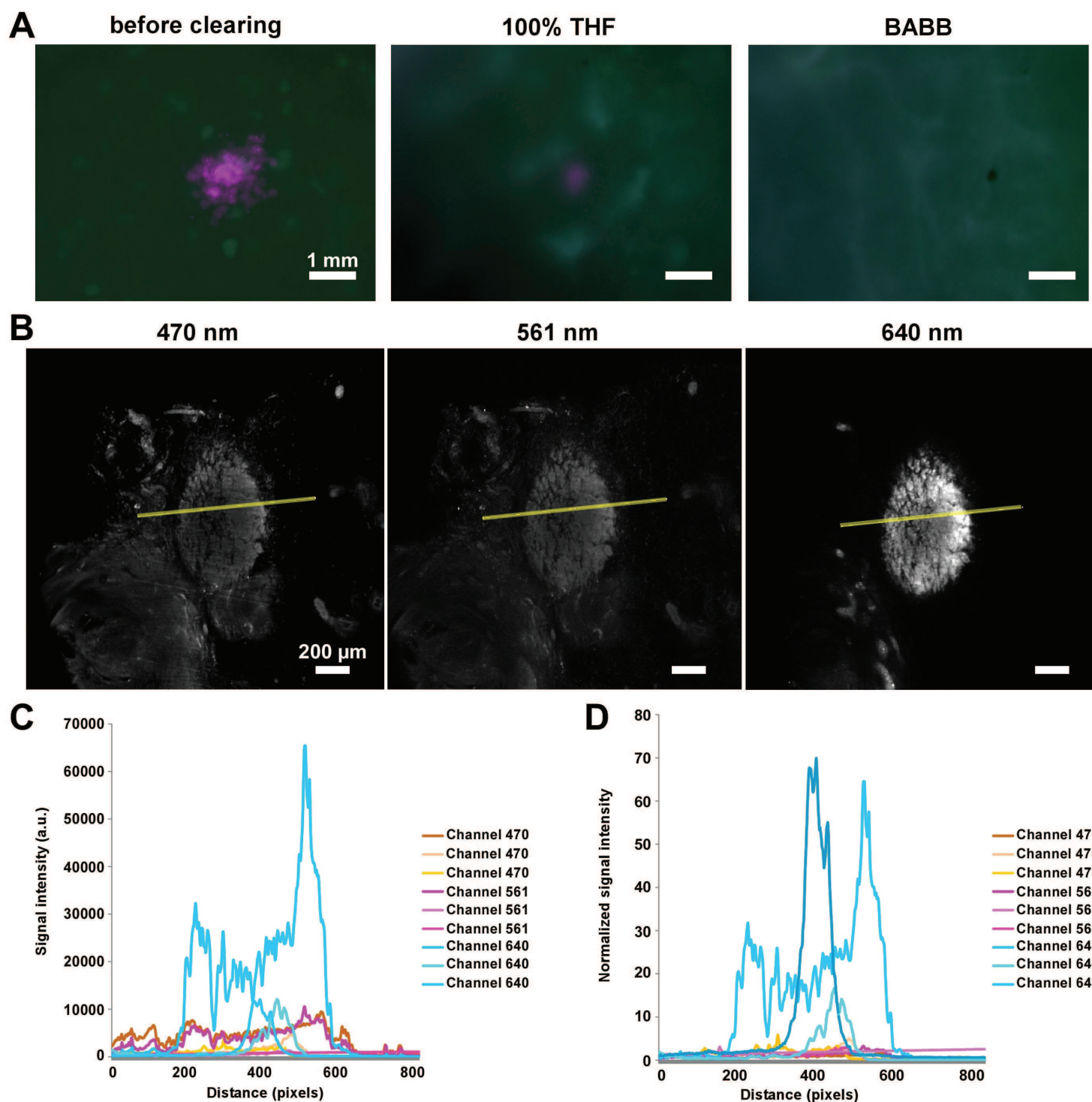




**Figure S5; related to Figure 5**

### Bioluminescence imaging of different cancer models

(A) Bioluminescence images with normal and long exposure from ventral and dorsal views of a mouse collected 21 days after intracardial (i.c.) injection of MCF-7 ER positive breast cancer cells. (B) Bioluminescence images of a mouse collected 10 days after intracardial injection of H2030-BrM3 lung cancer cells. (C) Bioluminescence images of a mouse collected 14 days after pancreatic injection of R254 cancer cells. Note that the C57BL/6 mouse line used in this model has black fur and therefore a different appearance in overlaid photographic/bioluminescence images compared to the other mouse strains. (D-F) Bioluminescence images of the time-course experiments shown in Figure 5. The mice were intracardially injected with MDA-MB-231 breast cancer cells and sacrificed 2 days, 6 days and 14 days post injection respectively.



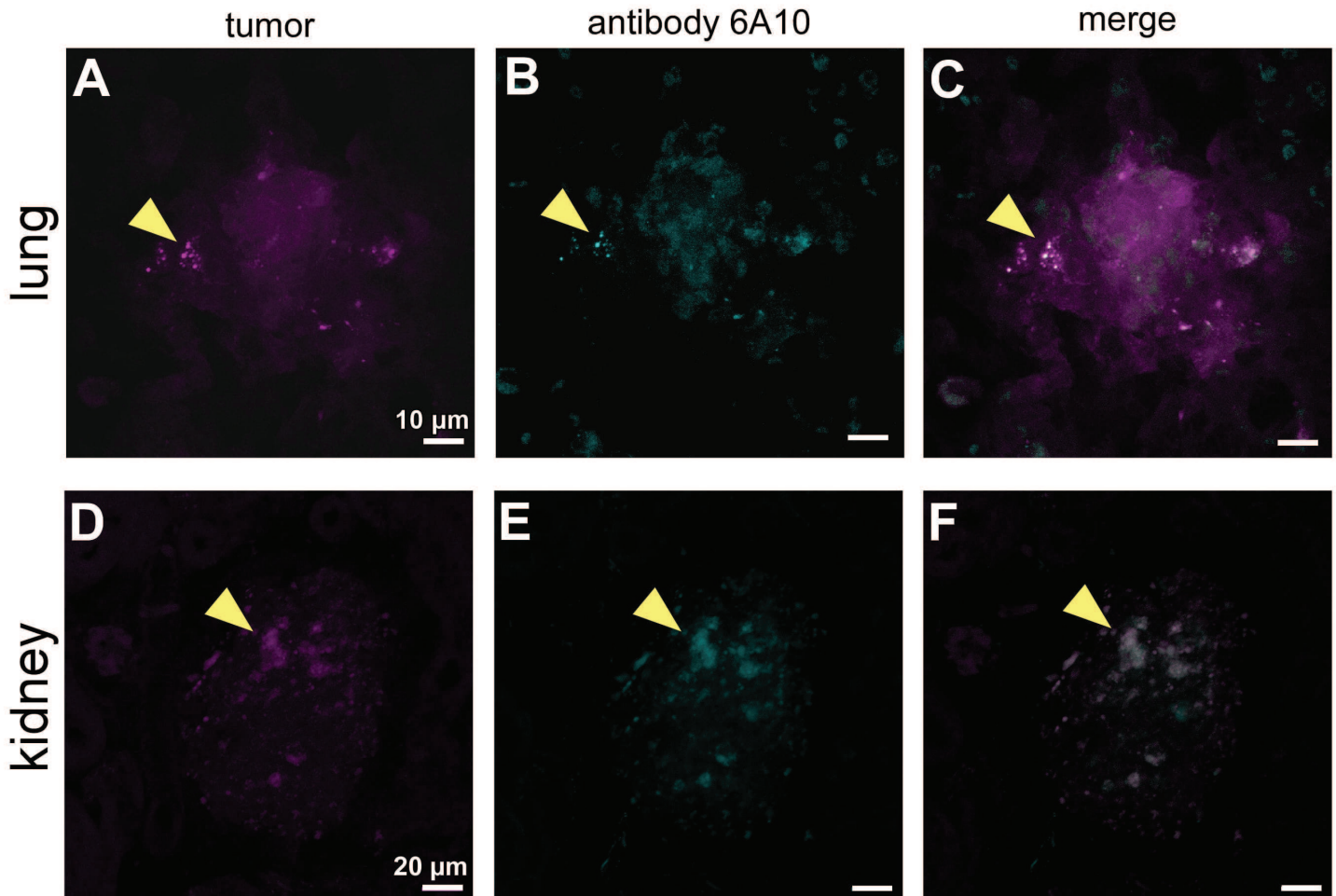
**Figure S6; related to Figure 6**

**Elimination of endogenously expressed mCherry signal from tumors after vDISCO**

(A) Tumor metastases in lungs from a mouse transplanted with MDA-MB-231 cells in the mammary fat pad were imaged with a fluorescence stereomicroscope before and after vDISCO clearing, showing that the endogenously expressed mCherry signal was eliminated after the THF and BABB incubation steps. (B) Light-sheet microscopy images of primary tumor with background fluorescence imaged in the green channel (ex: 470 nm, left), mCherry signal in the red channel (ex: 561 nm, middle), and the enhanced mCherry signal (Atto647N) in the far-red channel (ex: 640, right) after vDISCO clearing. (C) Signal intensity profiles along the yellow lines in panel B were plotted: Channel 470 (orange), Channel 561 (magenta) and Channel 640 (cyan) (n=3 mice). (D) Normalized fluorescence signal profiles of the data in (C), showing that the endogenous mCherry signal was depleted to background levels after vDISCO clearing.

animal treated with antibody 6A10 conjugated to Alexa568

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## Figure S7; related to Figure 6

### Verification of antibody targeting in different organs by confocal microscopy

(A-F) Confocal images of metastases in the lung (A-C) and kidney (D-F) of a mouse transplanted with MDA-MB-231 cells (labeled with an anti-mCherry nanobody conjugated to Atto647N, magenta) and treated with therapeutic antibody 6A10 conjugated to Alexa568 (cyan). Examples of the colocalization of metastatic cells with the 6A10 antibody are indicated with yellow arrowheads (C and F).